

Differences in field-scale N₂O flux linked to crop residue removal under two tillage systems in cold climates

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Abstract

Residue removal for biofuel production may have unintended consequences for N₂O emissions from soils, and it is not clear how N₂O emissions are influenced by crop residue removal from different tillage systems. Thus, we measured field-scale N₂O flux over 5 years (2005–2007, 2010–2011) from an annual crop rotation to evaluate how N₂O emissions are influenced by no-till (NT) compared to conventional tillage (CV), and how crop residue removal (R–) rather than crop residue return to soil (R+) affects emissions from these two tillage systems. Data from all 5 years indicated no differences in N₂O flux between tillage practices at the onset of the growing season, but CT had 1.4–6.3 times higher N₂O flux than NT overwinter. Nitrous oxide emissions were higher due to R– compared to R+, but the effect was more marked under CT than NT and overwinter than during spring. Our results thus challenge the assumption based on IPCC methodology that crop residue removal will result in reduced N₂O emissions. The potential for higher N₂O emission with residue removal implies that the benefit of utilizing biomass as biofuels to mitigate greenhouse gas emission may be overestimated. Interestingly, prior to an overwinter thaw event, dissolved organic C (DOC) was negatively correlated to peak N₂O flux ($r = -0.93$). This suggests that lower N₂O emissions with R+ vs. R– may reflect more complete stepwise denitrification to N₂ during winter and possibly relate to the heterotrophic microbial capacity for processing crop residue into more soluble C compounds and a shift in the preferential C source utilized by the microbial community overwinter.

Abbreviations

CT = conventional tillage
DOC = dissolved organic C
MBC = microbial biomass C
NT = no-till
R+ = crop residue returned
R– = crop residue removed
SMN = soil mineral N.

Keywords: agroecosystems, carbon substrates, crop residue, denitrification, greenhouse gas emission, nitrous oxide, overwinter, residue removal, tillage

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Introduction

Crop residue is increasingly used as a feedstock source for biofuel production, a practice claimed to reduce global greenhouse gas emissions. The influence of residue

removal on N₂O emissions is often ignored in life cycle analyses for biofuel production, potentially leading to an overestimated benefit of biofuels to mitigate greenhouse gas emissions (Smith & Searchinger, 2012). Based on IPCC emission factors, it is estimated that 1% of N in crop residue is converted into N₂O (IPCC, 2007), indicating that crop residue removal will consistently result in reduced N₂O emissions. However, studies have shown N₂O emissions to increase (Hao *et al.*, 2001; Yao *et al.*, 2013), decrease (Mutegi *et al.*, 2010; Abalos *et al.*, 2013; Jin *et al.*, 2014), or remain unaffected (Shan & Yan, 2013) due to crop residue removal. This emphasizes the

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uncertainty in predicting how crop residue removal influences N₂O emissions; clear information is required because N₂O depletes the ozone layer (Ravishankara *et al.*, 2009) and is a potent greenhouse gas with a global warming potential 298 times higher than CO₂ per mass (IPCC, 2007). Agriculture represents the largest source of anthropogenic N₂O emissions, primarily attributed to field cropping (Montzka *et al.*, 2011; Reay *et al.*, 2012). As agriculture is expected to expand and intensify for food and biofuel production, particularly in mid-latitude and cold climates (IPCC, 2014), it is imperative that research characterizes in detail the influence of crop residue management on N₂O emissions.

Regions across mid-latitude and cold climates contain the greatest extent of global agricultural land cover (Foley *et al.*, 2005; Ramankutty *et al.*, 2008), characterized by long winters when the soil is frozen or snow-covered for 5–6 months of the year. Despite these seemingly harsh conditions, 39–90% of annual N₂O emissions can occur overwinter (van Bochove *et al.*, 2000; Teepe *et al.*, 2000; Jayasundara *et al.*, 2007; Yanai *et al.*, 2011; Abalos *et al.*, 2015). Upon soil thawing, the physical release of N₂O trapped under ice cover or at depth in unfrozen soils has been often considered as the primary mechanism of overwinter N₂O flux (Bremner *et al.*, 1980; Burton & Beauchamp, 1994; Teepe *et al.*, 2001; Koponen *et al.*, 2004), due to the long-standing assumption that microbes are inactive overwinter due to low soil temperatures. Yet, recent studies have demonstrated evidence for *de novo* denitrification as the primary mechanism for N₂O emissions overwinter and during spring thaw (Wagner-Riddle *et al.*, 2008; Németh *et al.*, 2014), emphasizing the importance of microbiological processes at low temperatures. Evidence for active (or even peak) microbial biomass and respiration overwinter in cold regions has indeed been documented (Brooks *et al.*, 1996, 2005; Schimel *et al.*, 2004), but these studies were focused on arctic or subarctic ecosystems and very little is known for overwinter microbial activity in mid-latitude agroecosystems. Recent research by Németh *et al.* (2014) has characterized changes in soil microbial communities overwinter in contrasting tillage and residue management cropping systems and demonstrated that changes in N₂O flux were correlated with changes in bacterial nitrifier/denitrifier gene community composition. Because crop residue removal influences the soil heterotrophic community (Németh *et al.*, 2014), it also likely impacts N₂O dynamics, but more research is needed to characterize the effect. No studies have been conducted overwinter and over multiple seasons to fully characterize the influence of crop residue removal on N₂O emissions.

The N₂O response to crop residue removal may interact with tillage practices (Mutegi *et al.*, 2010) such that

residue removal may have more or less impact on N₂O flux depending on no-till vs. conventional tillage systems. But, very little is known on the topic because most studies have compared N₂O from contrasting tillage systems without manipulating crop residue removal effects. Based on studies which have returned crop residue to soils, no-till is commonly recommended as a best management practice to conserve water, reduce soil erosion, and organic matter losses, and no-till may reduce greenhouse emissions from soils when practiced over the long term (Six *et al.*, 2004). However, the effect of tillage on N₂O emissions is variable because it influences numerous soil biophysical factors that affect N₂O flux. No-till can increase N₂O emissions by reducing soil aeration, increasing soil C and N, and soil moisture within the surface layers, leading to higher denitrification rates compared with tilled soils (Rochette, 2008); alternatively, no-till can improve soil structure, lower soil temperature, and reduce N₂O loss (Six *et al.*, 2002; Venterea *et al.*, 2011). Consequently, some studies have found no difference or lower N₂O emissions from soils under no-till vs. conventional tillage (Parkin & Kaspar, 2006; Gregorich *et al.*, 2008; Rochette, 2008; Rochette *et al.*, 2008) while others have found higher N₂O emissions with no-till (Ball *et al.*, 1999; Chatskikh & Olesen, 2007). At Elora, Ontario, Wagner-Riddle *et al.* (2007) found that best management practices (including no-tillage) lowered N₂O emissions compared to conventional practices, but the comparison of no-till to conventional tillage was confounded by the use of reduced fertilizer rates in the best management treatment. These above-mentioned studies and the review by van Kessel *et al.* (2013) have demonstrated that N₂O emissions relate to the interaction of no-till management with factors such as soil properties, climate, and N fertilizer applications, but it is not clear how N₂O emissions are affected when crop residue is removed from no-till vs. conventional systems.

It was hypothesized that agricultural practices which reduce fluctuations in soil temperature overwinter will also reduce N₂O emissions, for example, by insulating the soil with snow trapped by un-tilled surface-placed crop residues (Wagner-Riddle *et al.*, 2007). A possible mechanism for why greater fluctuations in soil temperature increase *de novo* denitrification is that the freeze-thaw event causes microbial cell lysis (Schimel & Klein, 1996), which consequently increases the substrate available for denitrification, leading to N₂O loss. If this hypothesis is true, then removing crop residues and their associated insulation effect would actually increase N₂O loss compared to crop residues returned to soils. However, crop residue removal would also remove a C and N source for denitrification, so it is not clear how N₂O emissions would be affected. Field research of N₂O emis-

sions from different crop residue management practices over multiple years and seasons is needed, so accurate predictions can be made for how crop residue removal will influence N₂O flux, and for informing policy and recommendations on best management practices.

We address the research questions: (1) does conventional tillage have greater N₂O emissions than no-tillage overwinter and at the onset of the growing season? (2) how does removing crop residues after harvest influence N₂O emissions in no-till and conventionally tilled soils? Because peak N₂O loss events are sporadic throughout the year, micrometeorological methods for continuous and intensive sampling are best for fully capturing the N₂O emission events (Wagner-Riddle *et al.*, 2007). Thus, we employed micrometeorological techniques to obtain year-round field-scale N₂O measurements over 5 years (2005–2007, 2010–2011) from an agroecosystem trial in Ontario, Canada, to address our research questions.

Materials and methods

Experimental design

The experiment described herein is a continuation of a long-term study established in 2000 at the Elora Research Station, Ontario, Canada (43°38'N 80°25'W, elev., 376 m) as described previously (Jayasundara *et al.*, 2007; Wagner-Riddle *et al.*, 2007; Németh *et al.*, 2014). The soil at the site is classified as an imperfectly drained Guelph silt loam (29% sand, 52% silt, 19% clay) and experiences a moist mid-latitude climate with cold winters (Koeppen classification). Readers are referred to the previous publications for detailed site and soil descriptions.

Four field-scale plots (1.5 ha each) were located within an aerodynamically homogeneous 30-ha area, a requirement for the micrometeorological measurements, which provide spatially integrated fluxes over a large footprint. The crop rotation from 2000 to 2004 was corn (*Zea mays* L.), soybean (*Glycine max* L.), winter wheat (*Triticum aestivum* L.), corn, soybean (Table 1). Experimental treatments were established for (1) a conventional management practice (CP) vs. (2) a best management practice (BMP), each with two replicate plots (Wagner-Riddle *et al.*, 2007). Briefly, the conventional system included N fertilizer applied at the recommended rates of 90 kg N ha⁻¹ for winter wheat and 150 kg N ha⁻¹ for corn, and fall moldboard plowing, while in the BMP system N fertilizer applications were adjusted based on soil-test N and the soil was untilled. Measurements from 2000 to 2004 were previously published (Jayasundara *et al.*, 2007; Wagner-Riddle *et al.*, 2007). Here, we report measurements taken from (1) 2005 to 2007 (corn–soy–corn rotation) and from (2) 2010 to mid-2011 (soy–winter barley (*Hordeum vulgare* L.) (Table 1)). From 2005 to 2007, plots under CP and BMP received the same amount of N fertilizer (134 and 98 kg N ha⁻¹ of urea) broadcast at corn planting on May 11, 2005 and May 7, 2007, respectively. No N fertilizer was applied for soybean production in 2006. During this time period, the

two systems were differentiated only by tillage regime (conventional tillage (CT) vs. no-till (NT)), enabling us to address research question (1). After harvest in 2008 to 2010, crop residue was removed (R–) in one CT and one NT plot, and crop residues returned (R+) in one CT and one NT plot (Németh *et al.*, 2014). In the CT plots, the crop residues were mulched with a flail mower to facilitate residue incorporation by disk cultivation. The crop residue was removed as silage by cutting corn in November 2008 and 2009, and soybean stalks 5 cm above the soil with a silage cutter on September 27, 2010. After soybean harvest, winter barley was planted on October 1, 2010. No N fertilizer was applied for soybean production in 2010; fertilizer was broadcast at 98 kg N ha⁻¹ on April 14, 2011 for winter barley. The 2010 data reported here refer to the winter period following corn residue removal in fall 2009 and to the soybean growing season that followed (May–September 2010). The 2011 data refer to the winter period following soybean removal in fall 2010 and the growing season of winter barley (October 2010 to June 2011). The contrasting residue

Table 1 Mean crop yield, total biomass, and crop residue for harvests pertinent to the study periods 2005–2007 and 2010–2011 from conventionally tilled (CT) and no-till (NT) systems with crop residue returned after harvest (R+) vs. removed (R–)

		Mg ha ⁻¹		Estimated crop residue returned (+) or removed (–)
		Grain yield	Total biomass	
2004 (soybean harvest)				
CV		3.83	8.32	4.99
NT		3.59	8.57	5.45
SE		1.08	1.76	
2005 (corn harvest)				
CV		7.78	15.7	7.95
NT		8.45	17.4	8.90
SE		0.24	0.46	
2006 (soybean harvest)				
CV		3.36	8.78	5.43
NT		3.92	10.3	6.34
SE		0.16		
2009 (corn harvest)				
CT	R+	8.30	19.1	10.8
NT	R+	8.90	16.5	7.60
CV	R–	8.40	17.2	–8.80
NT	R–	9.50	19.1	–9.60
SE		0.50	1.10	
2010 (soybean harvest)				
CT	R+	2.59	7.72	4.87
NT	R+	2.91	7.87	4.96
CV	R–	2.83	7.64	–4.82
NT	R–	2.71	7.34	–4.62
SE				

Italicized values are estimated values using average soybean harvest index of 0.37 (index derived from 2004 soybean data).

management treatments (R– vs. R+) under CT and NT practices enabled us to address research question (2).

Crop yield measurements

At harvest, plants from six 1.5 m² quadrants were collected each plot. Fresh and dry biomass weights were determined (48 h at 50 °C). Crop yield and crop residue measurements from the experimental period of study are reported in Table 1.

N₂O flux measurements

The flux gradient method was used to obtain the vertical flux of N₂O for each plot (F_{N_2O}) (Wagner-Riddle *et al.*, 1996). A sampling tower was established in each field, and air was continuously directed to a sampling manifold located in an instrumentation trailer via a vacuum pump (Wagner-Riddle *et al.*, 2007; Németh *et al.*, 2014). Half-hourly averages of N₂O concentration differences between the upper and lower sampling heights were obtained using a tunable diode laser trace gas analyzer (TGA 100, Campbell Scientific Inc., Campbell Scientific Inc., Logan, UT, USA) located in the instrumentation trailer. Sonic anemometers were used to obtain the friction velocity and the integrated Monin–Obukhov similarity functions for heat, and cup anemometers were used to estimate friction velocity (u^*) when sonic anemometer data were not available (Wagner-Riddle *et al.*, 2007). Each plot had a maximum of 12 half-hourly flux values per day, as air sampling was sequentially timed among the four plots. The half-hourly data from each plot were filtered according to fetch, windspeed, and instrument diagnostic criteria described in Wagner-Riddle *et al.* (2007). Daily flux means were calculated by averaging half-hourly F_{N_2O} values using a minimum of two data points.

For all 5 years, the 0–120 days measurements were partitioned from the 120–180 days measurements to evaluate dynamics during the overwinter period separately from that during the onset of the growing season (which included N fertilization events at planting), respectively. Complete year-round data were available for each year except for 2007 and 2011 where summer and fall emission data were missing due to instrument failure. Micrometeorological data were not collected in 2008 or 2009.

Supporting data

Air temperatures were obtained from the Environment Canada weather station located approximately 200 m from the experimental plots, and snow depth on the ground was measured on each plot at several points (at least five) using a ruler. Soil temperature was measured at 5 cm depth hourly using thermistors (Model #107, Campbell Scientific) within each field. Also within each field, volumetric liquid water content (m³ m^{−3}) was continuously measured using Time Domain Reflectometry probes (Model CS616, Campbell Scientific Inc.), installed at approximately 10 cm depth. Half-hourly mean volumetric soil water content and soil temperature were recorded with a datalogger (21X Campbell Scientific Inc., Edmonton, AB).

To focus-in on the processes influencing N₂O emissions during a freeze–thaw cycle, soil samples were collected in 2010 on March

4 (before spring thaw) and March 17 (after spring thaw). To account for spatial variability within the 1.5 ha plots, a systematic soil sampling design was used (Pennock *et al.*, 2007). Within each plot, soil was sampled along three (150 m) transects, eight subsamples were randomly collected at regular intervals along each transect and combined into a composite sample. Soil microbial biomass C was quantified by the chloroform fumigation method (Voroney *et al.*, 2007). Briefly, oven dried soils (80 g weight basis) were placed in a desiccator and fumigated with 30 mL chloroform, and incubated (25 °C for 10 days); fumigated and nonfumigated soils were extracted with 150 mL 0.5 M K₂SO₄ followed by shaking for 1 h; filtered extractant was analyzed using a TOC-5000 analyzer (Shimadzu, Kyoto, Japan). Soil microbial biomass C was determined by subtracting fumigated from nonfumigated C values using a conversion factor of $K_{ec} = 0.35$ (standard fraction of biomass C) (Voroney *et al.*, 2007). Dissolved organic carbon was determined by subtracting inorganic carbon from total organic carbon measured in filtered K₂SO₄ extractant of nonfumigated soils (Horwath & Paul, 1994; Adair & Burke, 2010). Exchangeable NH₄⁺-N and NO₃[−]-N were extracted from soil samples with 2 M KCl, and concentrations of NO₃[−]-N in soil KCl extracts were measured by copper cadmium reduction to nitrite and of NH₄⁺-N by the indophenol blue method (Maynard *et al.*, 2007). Soil NH₄⁺-N and NO₃[−]-N were summed for quantifying soil mineral N (SMN).

Statistical analysis

The N₂O fluxes had log-normal distributions that were highly skewed (e.g., reverse J shaped). Thus, treatment comparisons were tested based on the Wilcoxon signed-rank test (Steel *et al.*, 1997) based on daily N₂O means using PROC NPAR1WAY in SAS 9.4 (Statistical Analysis System, version 9.3; SAS Institute, NC, USA). This statistical test was performed using only measured values (no interpolated daily means were used).

Results

Majority of total annual N₂O emissions occur during first 180 days of the year

Based on the annual 2005, 2006, and 2010 data, 28–77% of total annual N₂O emissions occurred overwinter during the first 120 days of the year, while 8–42% was emitted at the onset of crop production from 120 to 180 days (Fig. 1). Thus, the majority of cumulative N₂O emissions per year (i.e., 36–97%) was produced during the first 180 days of the year (Fig. 1).

N₂O emissions highest overwinter after soybean harvest and lowest during soybean growing season

During the 0–120 days period, which was prior to N fertilizer applications to corn, higher levels of N₂O flux were observed in 2005 and 2007 vs. 2006 (Fig. 2). The emission episodes in 2005 and 2007 followed soybean

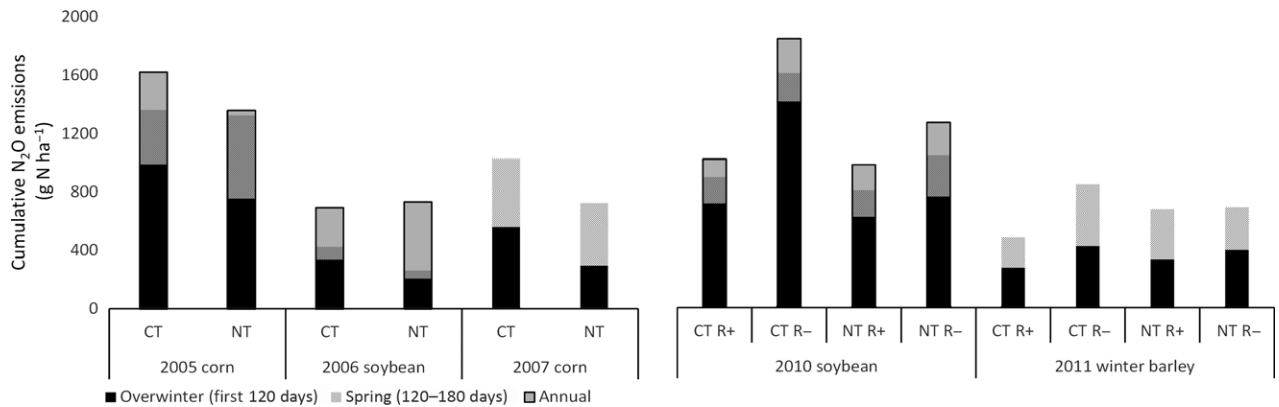


Fig. 1 Cumulative annual, overwinter, and spring N₂O flux data from 2005–2007 and 2010–2011 from 1.5 ha plots under conventional tillage (CT) and no-till (NT) with crop residue returned after harvest (R+) or removed (R-). Note that crop residue treatments were applied to corn in fall 2009 (for 2010 soybean) and soybean in fall 2010 (for 2011 barley). Data >180 days were missing for 2007 and 2011, and hence, annual emissions could not be estimated.

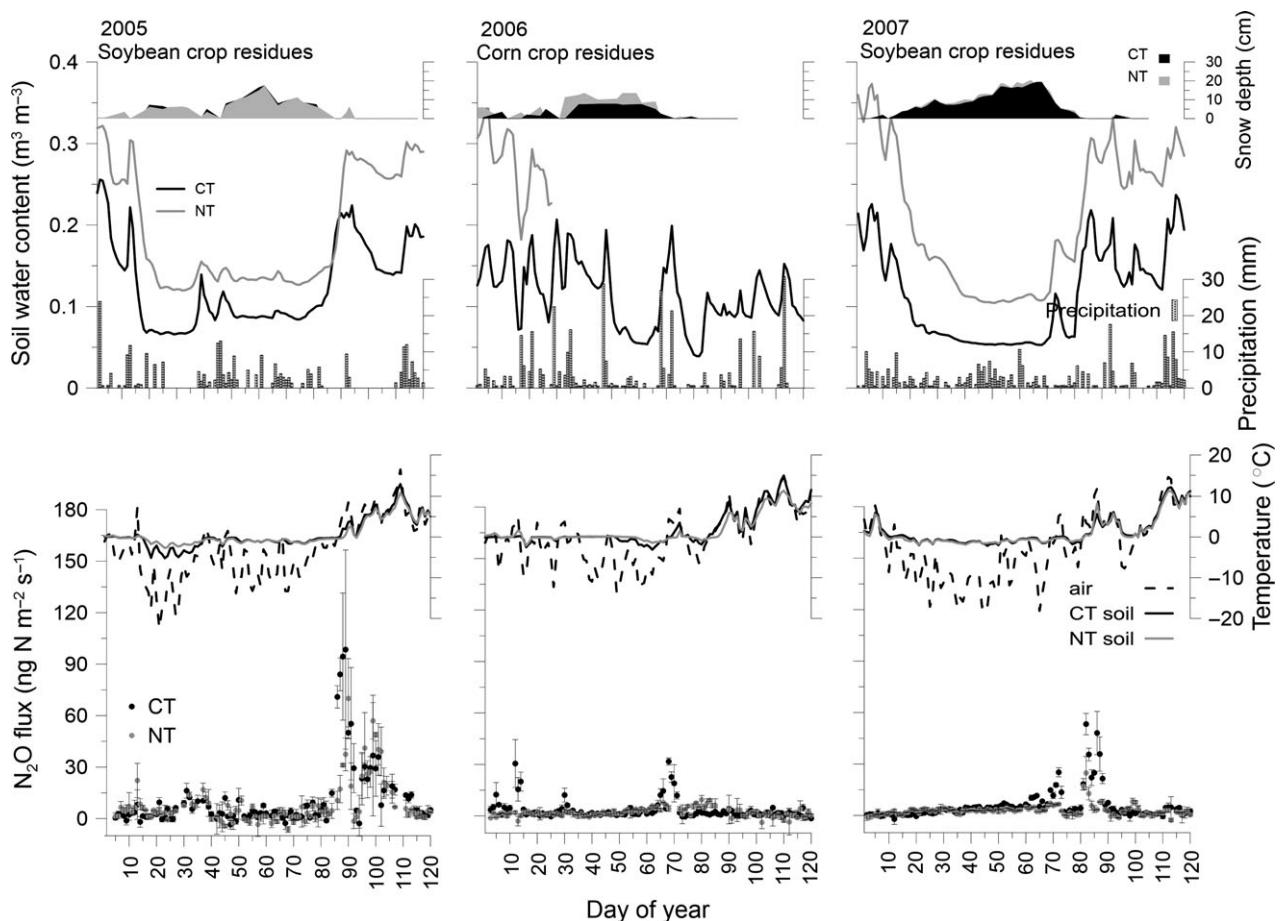


Fig. 2 Daily mean N₂O flux, soil temperature (5 cm depth), volumetric soil water content (10 cm depth), air temperature, snow depth, and precipitation during the overwinter period (days 0–120) from 1.5 ha plots under conventional tillage (CT) and no-till (NT) ($n = 2$).

crop residue addition to the soil in fall 2004 and 2006, which provided a readily mineralizable source of N. In contrast, the overwinter period in 2006 included corn

crop residues (a less mineralizable source of N as soybean residues), and the winter conditions during 0–120 days period in 2006 were less cold than 2005 or

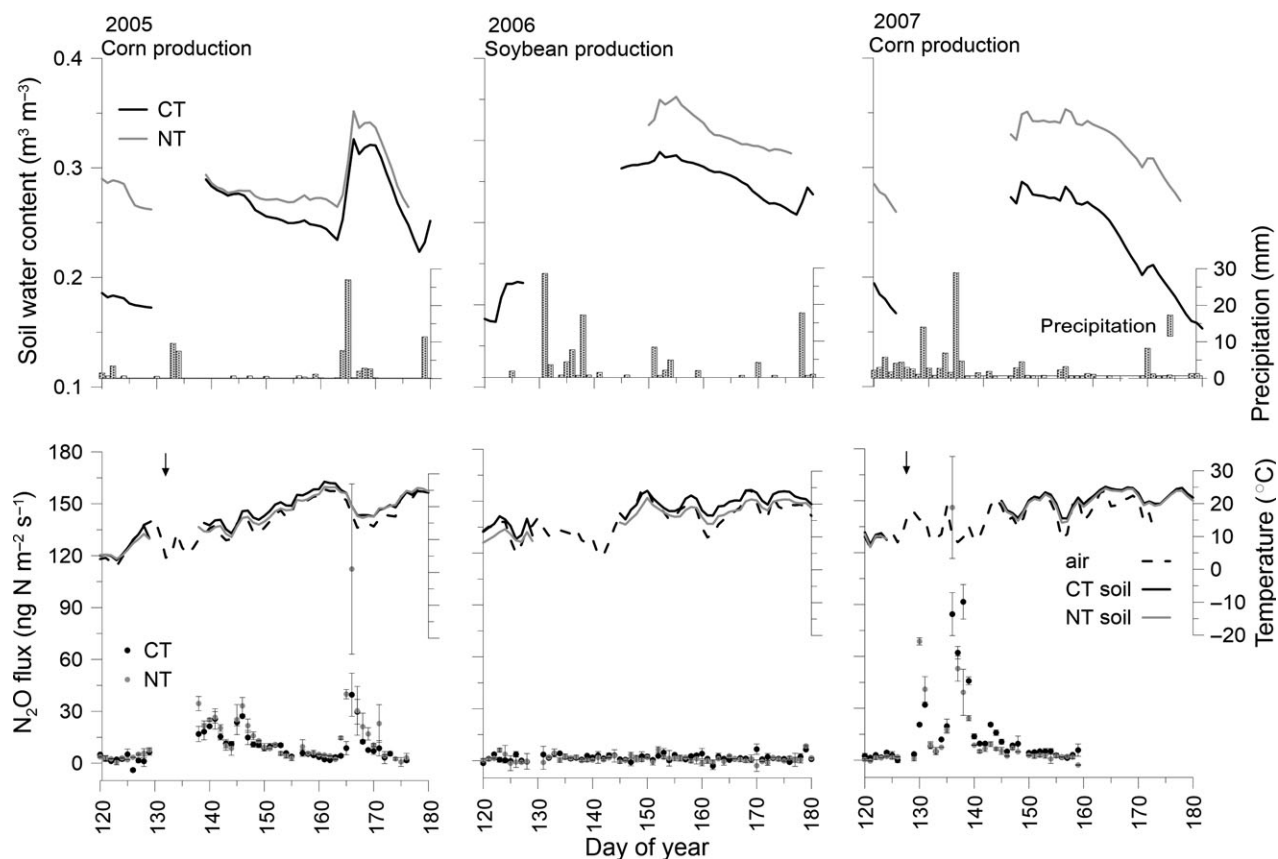


Fig. 3 Daily mean N_2O flux, soil temperature (5 cm depth), volumetric soil water content (10 cm depth), air temperature, and precipitation during the spring period (days 120–180) from 1.5 ha plots under conventional tillage (CT) and no-till (NT) ($n = 2$). Arrows indicate timing of N fertilizer application at crop planting.

2007 due to 86–135 fewer days with soil temperatures (5 cm depth) below 0°C and due to higher average soil temperatures by 0.6 – 1.1°C (Fig. 2).

N_2O emissions higher with conventional vs. no-till overwinter, but not at onset of growing season

Overwinter (0–120 days period) the N_2O emissions episodes corresponded to soil thawing and snow melt periods and typically lasted <2 weeks (Fig. 2). The CT system had significantly higher N_2O emissions compared to NT overwinter (Fig. 2, Table 2). The magnitude of peak overwinter N_2O flux was 1.4, 6.3 and 2.1 times greater from CT than NT in 2005, 2006, and 2007, respectively (Fig. 2). The differences in N_2O emissions between tillage systems overwinter corresponded to differences in soil moisture and temperature dynamics. The lower liquid soil water content under CT compared to NT from 2005 to 2007 (Figs 2 and 4) indicates a greater degree of soil freezing in the CT soils. Also, the CT soil had a slightly higher probability of experiencing soil temperatures below 0°C compared to NT (Fig. 4). Thus, the surface-placed crop residue in the NT systems

combined with the slightly deeper snow depth (Fig. 2) appeared to lower the intensity and variability in soil freezing overwinter by acting as insulation, supporting our prediction that agricultural practices which provide thermal insulation to the soil (i.e., surface-placed crop residues with NT practices) reduce N_2O emissions overwinter.

Regardless of the tillage regime, N_2O flux was relatively lower at the onset of the soybean growing season in 2006 compared to the onset of the corn growing season in 2005 and 2007 (Fig. 3); the result was attributable to lack of N fertilizer applications for the soybean crop in 2006. High N_2O emissions during the 120–180 days period were associated with N fertilizer applications and precipitation events (Fig. 3). During this period which encompassed N fertilizer applications, the N_2O flux from NT was not significantly different than from CT (Table 2), although the peak flux was 1.5–2.8 times greater with NT compared to CT in 2005 and 2007 (Fig. 3). From 2010 and 2011, N_2O emissions followed a similar trend as from 2005 to 2007, with significantly greater N_2O flux from CT vs. NT systems overwinter but not at the onset of the growing season (120–180 days per-

Table 2 Wilcoxon nonparametric test comparing N₂O daily flux from conventionally tilled (CT) and no-till (NT) systems during winter and spring periods from 2005 to 2007 and 2010 to 2011

	Overwinter period (0–120 days)		Spring period (120–180 days)	
	CT	NT	CT	NT
2005–2007				
Mean Wilcoxon score	703	654	298	284
Median daily flux (ng N m ⁻² s ⁻¹)	2.56	2.43	3.54	2.96
Mean daily flux (ng N m ⁻² s ⁻¹)	6.39	4.32	7.45	8.38
Sample size	683	673	289	291
Z value	–2.30		1.01	
Pr > Z	0.021		0.313	
2010–2011*†				
Mean Wilcoxon score	406	347	170	172
Median daily flux (ng N m ⁻² s ⁻¹)	5.36	4.4	3.57	3.39
Mean daily flux (ng N m ⁻² s ⁻¹)	8.94	6.44	7.1	7.4
Sample size	367	383	167	174
Z value	3.73		–0.1357	
Pr > Z	0.0002		0.8921	

Bold values indicate significant difference between tillage treatments at $p < 0.05$.

*For 2010–2011, NT and CT mean comparison included data from plots with residue removed and residue returned.

†For 2011, the data were partitioned as 0–100 days and 100–180 days for overwinter and spring, respectively, so that only the spring period reflects N₂O response after N fertilizer was applied on day 104.

iod) (Table 2). The temporal variability in N₂O response to contrasting tillage regimes points toward a fundamental shift in the predominant mechanism responsible for N₂O emissions upon seasonal transition.

Removing crop residue increases N₂O emissions, especially with conventional tillage

Based on the 2010 and 2011 overwinter data, crop residue removal tended to increase N₂O flux (Fig. 5, Table 3) although significance ($P < 0.05$) was detected for the CT system only. At the onset of the growing season, R– increased N₂O flux relative to R+ at $P < 0.1$ from both CT and NT systems (Fig. 6, Table 3). Thus, a tillage × residue effect on N₂O flux appeared stronger overwinter than at the onset of the growing season.

Nitrous oxide emissions were relatively low overwinter in 2011 (Fig. 5) compared to 2010, but 2011 was differentiated due to deeper snow levels (Fig. 5) and the presence of an overwinter barley crop.

Discussion

Overwinter N₂O emissions represent the majority of total annual emissions regardless of tillage system or residue removal

Irrespective of tillage system or residue removal, 28–77% of total annual emissions occurred during the

overwinter period from January to March (Fig. 1). Like our study, others have found the majority of N₂O emission occurs during the nongrowing season in cold climates, (i.e., November–April) by representing 39–90% of the cumulative annual emissions (Wagner-Riddle *et al.*, 2007; Yanai *et al.*, 2011; Abalos *et al.*, 2015). The environmental conditions during this period generally increase the risk for N₂O loss for a variety of reasons, such as anaerobic soil conditions promoting denitrification (Wagner-Riddle *et al.*, 2007, 2008), physical release of N₂O trapped by ice or snow cover upon thawing (thus primarily influencing the timing of flux) (Risk *et al.*, 2013), increased availability of labile organic C and mineral N in soil (due to absence of plant N uptake, disaggregation of soil aggregates upon freeze–thawing, microbial lysis due to low temperatures) (Schimel & Clein, 1996), and increased N₂O:N₂ ratio due to environmental impacts on N₂O reductase activity (Németh *et al.*, 2014). Thus, the overwinter period should be considered when assessing the impact of residue management on N₂O emissions in cold climates.

Without overwinter or continuous year-round N₂O measurements, misleading results may be obtained. For example, Gregorich *et al.* (2008) concluded that soybean crops do not substantially contribute to annual N₂O emissions compared to other crops such as corn, yet no measurements were taken during the subsequent overwinter period (first 100 days of the year). Because the risk of N₂O emissions is higher during the senescence

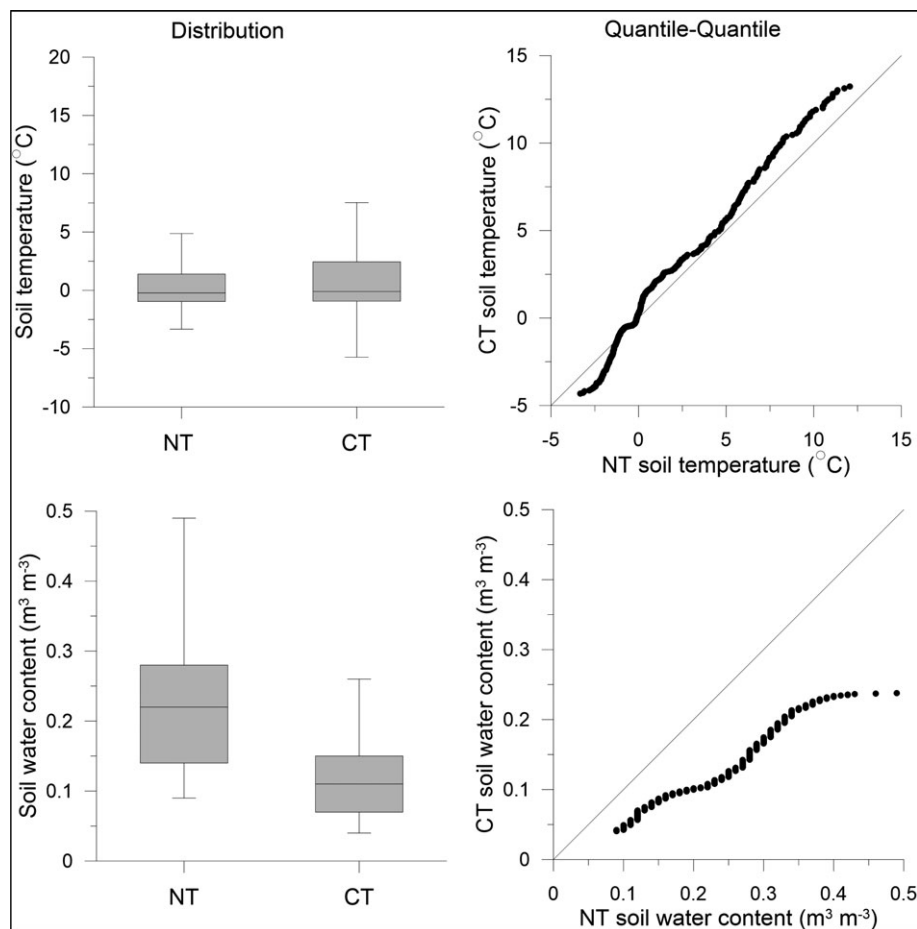


Fig. 4 Distribution (left) and quantile-quantile plot (right) for soil temperature and volumetric soil water content (10 cm depth) during the 0–120 days period overwinter (2005–2007) from 1.5 ha plots under conventional tillage (CT) and no-till (NT) ($n = 2$).

and decomposition of soybean crop residues rather than during soybean growth (Yang & Cai, 2005), it is necessary to measure overwinter N_2O flux following soybean harvest (Halvorson *et al.*, 2008). With discontinuous measurement techniques, there is a high chance of missing episodic N_2O flux events during winter and spring periods; thus, our results emphasize the importance of year-round continuous N_2O flux measurements.

Seasonal variation in N_2O response to tillage systems may indicate shift in mechanisms responsible for emissions

Tillage system did not influence N_2O emissions at the onset of the growing season (Fig. 3, Table 2) indicating that NT was not effective in mitigating N_2O emissions compared to CT practice at crop planting. At this same experimental site in Elora from 2000 to 2004, the NT treatment was formerly a BMP system and differentiated from the CT system by lower N fertilizer rates and no-till; during this period, the BMP system had the lowest N_2O emissions (Wagner-Riddle *et al.*, 2007). Because

the NT and CT systems from 2005 to 2007 were differentiated by tillage only (i.e., both had the same N fertilizer applications), our findings imply that the growing season results from Wagner-Riddle *et al.* (2007) were largely driven by the contrasting N fertilizer applications at planting (May–June). Among the two conservation management practices of no-till and reducing N fertilizer rates at preplant, the latter might be the more effective strategy for mitigating N_2O emissions at the onset of crop production in early spring. Numerous studies have shown N fertilizer application rates predominantly influence N_2O emissions (Baggs *et al.*, 2003; Malhi & Lemke, 2007; Sánchez-Martín *et al.*, 2008; Risk *et al.*, 2013).

Despite the lack of differences in N_2O emissions between tillage practices at the onset of the growing season, our micrometeorological data from all 5 years demonstrated that CT had higher N_2O emissions than NT overwinter (Table 2). Similarly, but using chamber methodology, Mutegi *et al.* (2010) observed significant tillage by season interactions on N_2O flux. A possible

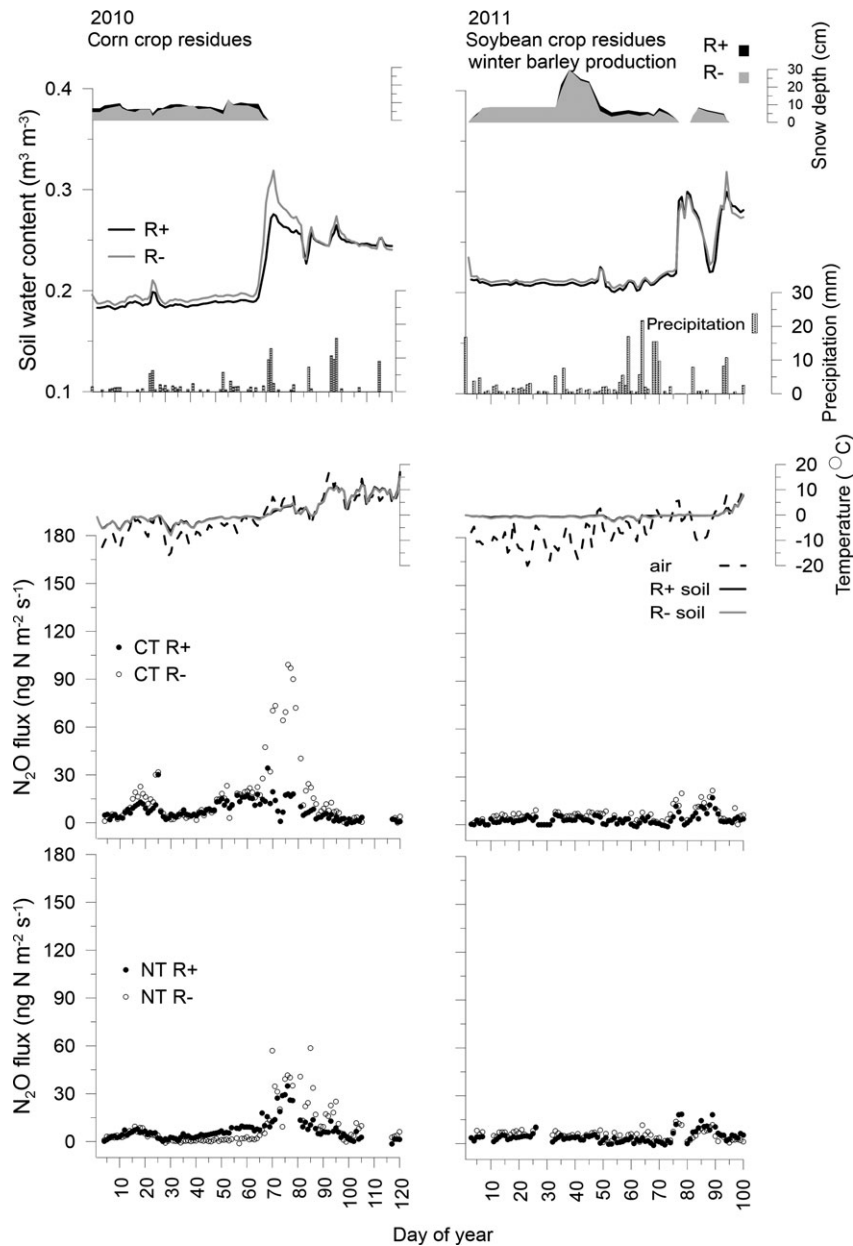


Fig. 5 Daily mean N₂O flux, soil temperature (5 cm depth), volumetric soil water content (10 cm depth), air temperature, snow depth, and precipitation during the overwinter period (days 0–120) from 1.5 ha plots under conventional tillage (CT) and no-till (NT) with crop residue returned after harvest (R+) or removed (R-) ($n = 1$). (For 2011, the data were partitioned as 0–100 days for the overwinter period so that it does not reflect the N₂O response due to the N fertilizer application on day 104).

explanation for the contrast in results between the seasons could be a shift in the pathway primarily responsible for N₂O production. Emissions via *de novo* denitrification might have prevailed overwinter, while N₂O loss via nitrification–denitrification may have dominated during spring after the urea N fertilizer was applied. Accordingly, the denitrifier community may not be as affected by cold temperatures as nitrifiers, leading to more obvious differences in the soil

microbial community structure associated with tillage systems overwinter (i.e., March thaw) than after N fertilizer events at spring planting (Smith *et al.*, 2010). Further, shifts in microbial denitrifier/nitrifier communities have been observed over time and between frozen and unfrozen soil conditions (Wertz *et al.*, 2013). Soil incubated at -1°C had greater N₂O emissions and denitrification than soils incubated above 0°C , a result which may be related to the restriction

Table 3 Wilcoxon nonparametric test comparing N₂O daily flux with crop residue returned after harvest (R+) vs. removed (R-) from conventionally tilled (CT) and no-till (NT) systems 2010 to 2011

	CT		NT	
	R+	R-	R+	R-
Overwinter period (0–120 days)				
Mean Wilcoxon score	160	210	193	191
Median daily flux (ng N m ⁻² s ⁻¹)	4.49	6.68	4.36	4.48
Mean daily flux (ng N m ⁻² s ⁻¹)	6.05	12.0	5.80	7.08
Sample size	189	178	192	191
Z value	4.50		-0.107	
Pr > Z	<0.0001		0.915	
Spring period (120–180 days)*				
Mean Wilcoxon score	74.1	93.3	80.5	94.2
Median daily flux (ng N m ⁻² s ⁻¹)	2.97	4.07	3.17	3.77
Mean daily flux (ng N m ⁻² s ⁻¹)	5.64	8.47	7.23	7.56
Sample size	81	86	85	89
Z value	-2.57		-1.79	
Pr > Z	0.010		0.074	

Bold values indicate significant difference between residue treatments at $p < 0.05$.

*For 2011 time periods, data were partitioned as 0–100 days and 100–180 days for overwinter and spring, respectively, so that only the spring period reflects N₂O response after N fertilizer was applied on day 104.

of N₂O reductases and/or accumulation of NO₂⁻ below 0 °C (Wertz *et al.*, 2013). In addition to shifts in microbial community structure and denitrification between seasons, a shift in preferential C source fueling microbial activity could explain our results.

Winter-adapted heterotrophic microbial communities grow at low temperatures (down to -5 °C) and primarily utilize easily degradable and simple C constituents (Schmidt & Lipson, 2004; Brooks *et al.*, 2005). After snowmelt and thaw, there is generally a die-off of the winter-adapted microbial community due to starvation and intolerance of higher soil temperatures, and thereafter, the summer-adapted microbial community utilizes more complex C inputs (Schmidt & Lipson, 2004). While the research by Brooks *et al.* (2005) and Schmidt & Lipson (2004) focused on subarctic temperate soils, this understanding of microbial community dynamics and activity can nevertheless be applied to temperate agroecosystems during winter. In our study, the active soil microbial community may have utilized a different source of C overwinter than during the growing season, resulting in differences in N₂O loss dynamics between winter (Fig. 2) and spring periods (Fig. 3).

The presence of unfrozen water in soil allows microbial activity to continue in cold winter conditions (Mikan *et al.*, 2002), and it is possible that heterotrophic microbial respiration persisted in the NT soils due to the lower degree of soil freezing compared to the CT practice (Fig. 4). Because water expands and takes up more space as it freezes, the lower extent of soil water freezing under NT might have resulted in better soil aeration overwinter

than under CT. Thus, a possible explanation for why NT was effective in reducing overwinter N₂O emissions in our study was that soil under NT experienced less anaerobic conditions than under CT, leading to less N₂O loss. However, the explanation could be more complex, because members of the soil bacterial community may have differential affinities for C sources overwinter, and C sources are likely impacted by tillage regime. While the CT system in our study consisted of moldboard plowing and disk cultivation, the intensity of tillage systems could also play a role in determining N₂O flux overwinter and warrants future research.

Understanding the effect and implications of crop residue removal on N₂O emissions

Our results challenge the assumption based on IPCC methodology (IPCC, 2007) that crop residue removal will result in reduced N₂O emissions. Consistent with our results, higher N₂O emissions were associated with crop residue removal upon soil thawing based on a study carried out in Canada where soils freeze overwinter (Hao *et al.*, 2001). Also in southeast China, residue removal increased N₂O emissions with peak flux occurring in December when minimum soil temperatures were below or at 0 °C (Yao *et al.*, 2013). However, removing crop residues lowered N₂O emissions in conventional till soils in Western Denmark (Mutegi *et al.*, 2010), but their soil did not freeze and mean temperatures never dropped below 2 °C. Abalos *et al.* (2013) found crop residue removal reduced N₂O

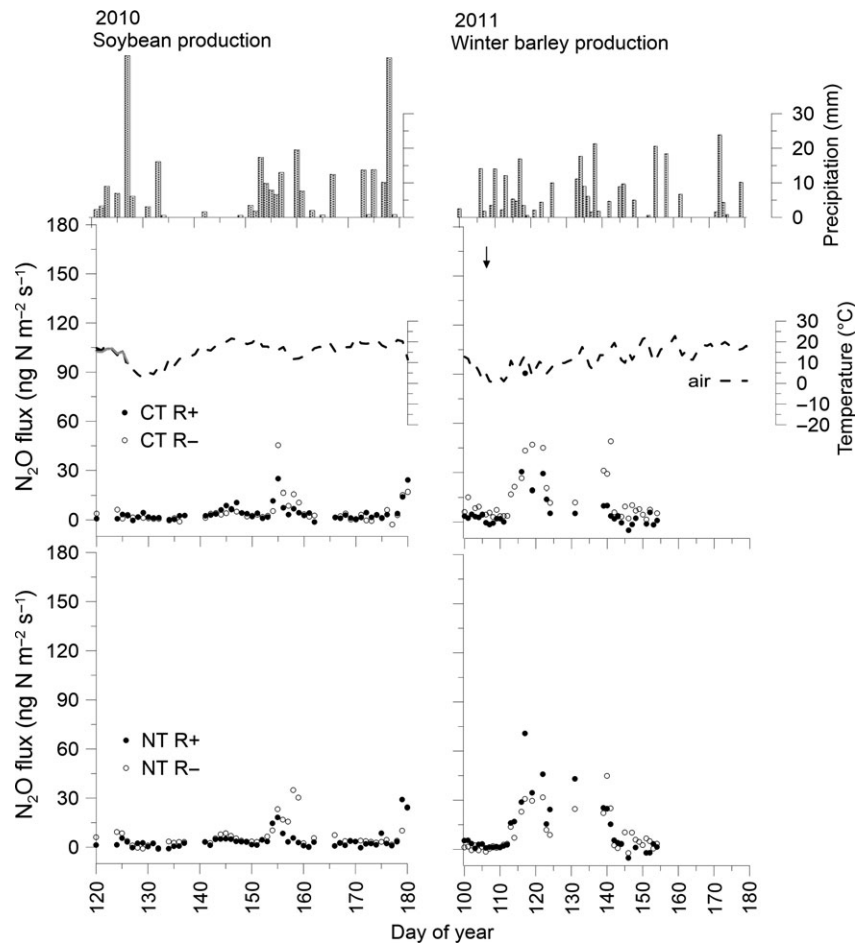


Fig. 6 Daily mean N₂O flux, air temperature, and precipitation during the spring period (days 120–180) from 1.5 ha plots under conventional tillage (CV) and no-till (NT) with crop residue returned after harvest (R+) or removed (R–) ($n = 1$). (For 2011, the data were partitioned as 100–180 days for the spring period so that it reflects the N₂O response due to the N fertilizer application on day 104).

emissions, but again, mean soil temperatures were above 0 °C in a Mediterranean climate. The results from Mutegi *et al.* (2010) and Abalos *et al.* (2013) were explained as such: returning crop residue to soils provided a source of C which promoted denitrification and N₂O loss; this seems very plausible in their case where soils were C limited and experienced limited freeze–thaw cycling, but the explanation may not apply to colder or more fertile soils. In climates which experience soil freezing, heterotrophs may preferentially utilize more labile C (Schmidt & Lipson, 2004; Brooks *et al.*, 2005) rather than crop residue-C overwinter and this shift in C source overwinter may contribute to discrepancies among studies in the literature. If crop residue removal promotes N₂O loss overwinter in climates which experience freeze–thaw cycling, as our study suggests, this would have negative implications for greenhouse gas emissions upon biomass removal and would lessen the benefit of uti-

lizing biomass as biofuels to reduce net greenhouse gas emissions.

It is worth acknowledging that the severity of overwinter conditions (temperature fluctuations, snow depth, and duration of snow cover) varies from location to location and year to year even within cold climate classifications. This type of variability across regions or yearly weather patterns likely has considerable influence on the relative residue removal effects on N₂O emissions (Jin *et al.*, 2014).

Previous studies have not found significant interactions between tillage system and residue removal on N₂O emissions (Baggs *et al.*, 2003; Malhi & Lemke, 2007), but these studies did not encompass the overwinter period. Mutegi *et al.* (2010) observed the N₂O response to crop residue removal interacted with tillage practices overwinter only and not at the onset of the growing season; our results were similar but the interaction between tillage and crop residue removal was more

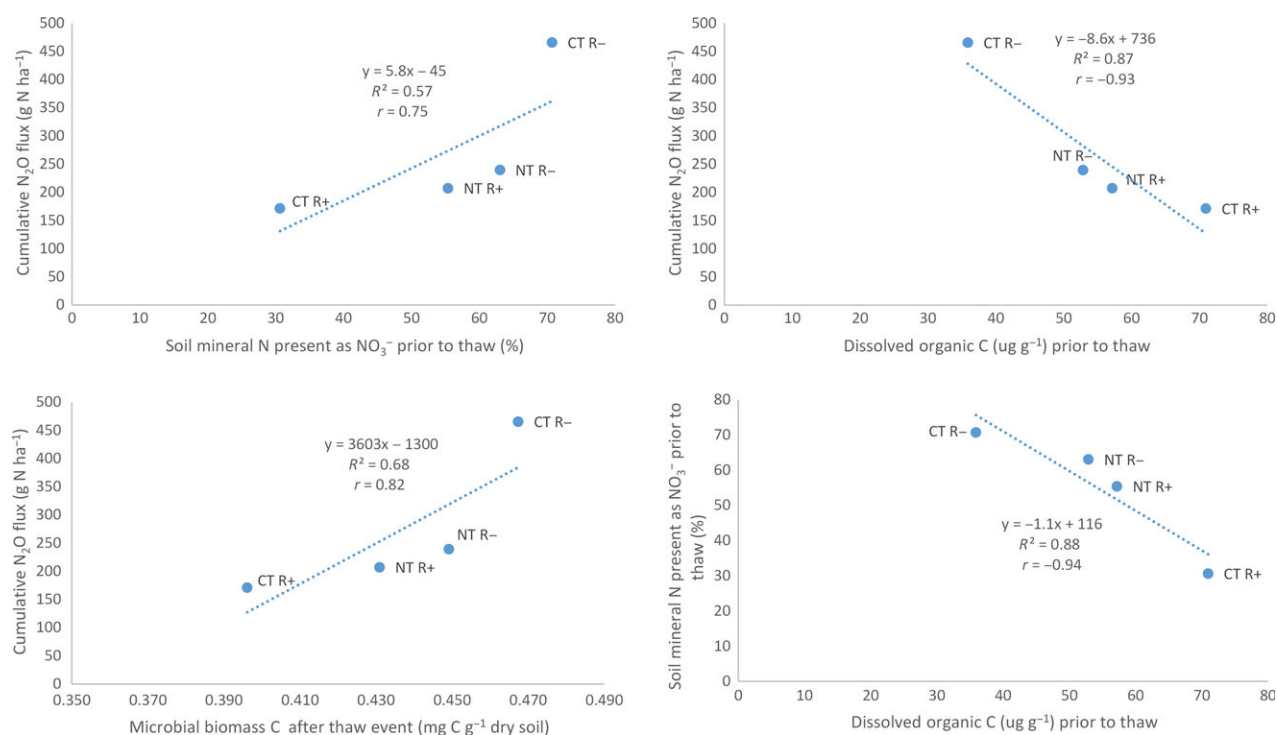


Fig. 7 Peak cumulative N₂O emissions in relation to soil variables (0–15 cm) over a 14 days thaw period from days 63 to 76 in 2010 from 1.5 ha plots under conventional tillage (CT) and no-till (NT) with crop residue returned after harvest (R+) or removed (R-) ($n = 1$).

distinct overwinter than during the growing season (Table 3). Possible reasons for why R- increased N₂O flux relative to R+ to a greater extent within the CT than the NT system could relate to more dramatic differences in soil temperature and moisture dynamics between tillage regimes overwinter than during spring, but there remain many unknowns in the understanding of environmental controls of N₂O release at temperatures around 0 °C (Butterbach-Bahl *et al.*, 2013). Thus, we stress the need for more research to identify the mechanisms which drive the interaction and to incorporate these mechanisms into process-based models for improved predictions of thaw-induced N₂O flux. This type of research would contribute to better emission factors for predicting the effect of management on greenhouse gas emissions.

Overwinter N₂O flux and the nature of heterotrophic activity: need for future research

Over the 14 days thaw period in 2010 (day 63 and 76; Fig. 5), cumulative N₂O emissions totaled 466, 239, 207, and 171 g N ha⁻¹ for CT R-, NT R-, NT R+, and CT R+, respectively (Fig. 7); the daily soil temperatures ranged from -1.8 to 3.8 °C, with 0.30 °C greater variation in soil temperature in the R- vs. R+ plots. Higher

cumulative N₂O emissions were strongly correlated to (i) the MBC level after the freeze-thaw cycle and (ii) proportion of SMN present as NO₃⁻ prior to the thaw event (Fig. 7). This would support N₂O flux via *de novo* denitrification rather than the physical release of trapped N₂O in ice films overwinter, a finding which agrees with the suggestion by Nemeth *et al.* (2014) and Wagner-Riddle *et al.* (2008). However, the total absolute concentration of soil NO₃⁻ or SMN was weakly correlated to cumulative N₂O loss ($R^2 < 0.18$ and $r < 0.43$), indicating that N₂O flux was not simply a function of absolute SMN availability for denitrification; thus, other factors were explored, such as DOC. (Soil NO₃⁻ and SMN ranged from 17–43 kg ha⁻¹ and 56–77 g ha⁻¹, respectively, in top 15 cm and thus did not limit denitrification rates.)

Interestingly, cumulative N₂O loss was strongly and negatively ($r = -0.93$, $n = 4$) related to DOC levels immediately prior to the thaw event in 2010 (Fig. 7); there was evidence for DOC consumption during the thaw period as DOC levels dropped by up to 13% on average from before to after the thaw event. While this finding highlights the importance of soluble C availability in regulating N₂O loss, it did not support the hypothesized mechanism responsible for increasing N₂O emissions: increased C substrate available (caused

by low temperatures and microbial lysis overwinter) will increase N₂O loss via denitrification. While many studies and models suggest that elevated soluble C levels lead to increased N₂O emissions by providing a C substrate for denitrification (Li *et al.*, 2005; Sanchez-Martin *et al.*, 2010), very few studies have collected DOC measurements or reported DOC data to support this hypothesis. As Berggren & Giorgio (2015) found, there is surprisingly little understanding of how DOC source and composition regulate patterns in microbial C utilization; yet the source and composition of DOC strongly influences bacterial metabolism.

The decomposition of organic matter in soil may proceed as a sequence where plant-derived material is converted into microbial-derived material, a process regulated by environmental and physical factors (Schmidt & Lipson, 2004; Grandy & Neff, 2008). Thus, relative differences in DOC levels among treatments overwinter could reflect the quantity of microbial-derived material or the breakdown of plant material into smaller soluble compounds. During winter, soil microbes may preferentially utilize more labile C sources (Schmidt & Lipson, 2004; Brooks *et al.*, 2005). With CT vs. NT and with R– vs. R+ in our experiment, decomposers might have had a relatively lower capacity to process crop residue into more soluble C compounds (i.e., transform crop residue-C and produce microbial-derived C) overwinter because of (1) the greater extent of soil freezing with CT vs. NT, and (2) less C input with R– vs. R+. Consequently, NT and R+ practices could have resulted in more labile soil C (i.e., DOC; Fig. 7) overwinter which facilitated more complete step-wise denitrification during anaerobic conditions (thereby reducing N₂O emissions) compared to CT or R– practices. In support of this mechanism, Németh *et al.* (2014) found that R+ and R– treatments did not differ in nitrite reductase (*nirS*) gene quantities; however, the nitrous oxide reductase (*nosZ*) gene quantities were greater with R+ than R–. Thus, Németh *et al.* (2014) concluded that lower N₂O emissions were a result of enhanced reduction in N₂O to N₂, rather than a decline in total NO₃[–] denitrified. Similarly, the addition of labile C sources to soil reduced emissions of NO and N₂O (Sánchez-Martín *et al.*, 2008). The *nirS* and *nosZ* communities may respond differently to organic C amendments, as the addition of plant residues had no significant impact on *nirS*-bearing denitrifiers but increased the abundance of *nosZ*-bearing denitrifiers (Henderson *et al.*, 2010).

Conditions supporting decomposition overwinter which influence the presence of labile organic C will also influence SMN dynamics and N₂O loss. We found DOC levels were inversely related ($r = -0.94$) to the proportion of SMN present as NO₃[–] immediately prior

to the overwinter thaw event in 2010 (Fig. 7). Thus, a related mechanism for reduced N₂O emissions with increased DOC could be N immobilization during aerobic conditions. As such, readily mineralizable organic C amendments have reduced N losses by causing N immobilization (Congreves *et al.*, 2013a,b, 2014). We recommend future research address how decomposition and DOC composition influence N₂O emissions and denitrification overwinter.

Conclusions

Data from all 5 years indicated no differences in N₂O flux between tillage practices at the onset of the growing season. However, the CT system had higher N₂O emissions than the NT systems overwinter (0–120 days). A possible explanation for the contrast in results between the overwinter period and the growing season period could be a shift in the pathway primarily responsible N₂O production. This new knowledge of the temporal variation in the effect of tillage system on N₂O highlights the importance of designing experiments with year-round measurements to better understand the impact of agricultural management on greenhouse gas emissions.

Nitrous oxide emissions were higher after removing crop residues compared to returning crop residues to the soil, but the effects were more marked under conventional tillage than no-till, and overwinter than during spring. This trend challenges the assumption based on IPCC methodology that crop residue removal will consistently result in reduced N₂O emissions, and more research is needed to better characterize, understand, and simulate the mechanisms responsible for this effect. The common assertion that harvesting crop biomass as an alternative fuel source is beneficial for reducing greenhouse gas emissions may be overestimated. Ultimately, the temporal effects of tillage and residue management on N₂O flux should be reconsidered in modeling efforts to better characterize how greenhouse gas emissions from soils in cold climates are influenced by agricultural practices.

Our results support the prediction that agricultural practices that provide thermal insulation to the soil (i.e., surface-placed crop residues) reduce N₂O emissions overwinter. The originally hypothesized mechanism for this effect was that a lower extent/variability of soil freezing reduces the magnitude/frequency of microbial cell lysis, consequently reducing substrate availability (i.e., DOC) for denitrification and N₂O loss (i.e., low DOC would relate to low N₂O loss). Yet in contrast, peak N₂O flux was inversely related to DOC levels during an overwinter thaw event ($r = -0.93$). Thus, decreased overwinter N₂O loss with NT vs. CT and with R+ vs. R– may be

due to more complete stepwise denitrification of NO_3^- to N_2 facilitated by higher DOC levels, possibly related to the heterotrophic microbial capacity to process crop residue into more soluble C compounds and a shift in the preferential C source utilized by the microbial community overwinter; we recommend future research investigate this process in detail.

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